

Data Evaluation Report on the acute dietary toxicity of Acetamiprid insecticide to the Bobwhite Quail (*Colinus virginianus*)**PMRA Submission Number 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090**
EPA MRID Number 44651860

Data Requirement: : PMRA DATA CODE: 9.6.2.4
EPA DP Barcode:
OECD Data Point: IIA 8.1.2
EPA Guideline: US EPA Subdivision E Guideline 71-2**Test material:** NI-25 **Purity (%):** >99%
Common name: Acetamiprid
Chemical name: N^1 -[(6-chloro-3-pyridyl)methyl]- N^2 -cyano- N^1 -methylacetamidine
IUPAC: (*E*)- N^1 -[(6-chloro-3-pyridyl)methyl]- N^2 -cyano- N^1 -methylacetamidine
CAS name: (*E*)- N -[(6-chloro-3-pyridinyl)methyl]- N^1 -cyano- N -methylethanimidamide
CAS No.: 160430-64-8
Synonyms: Pristine Brand RTU, Chipco Brand Tristar 70 WSP,
Adjust Brand 70 WP and Assail Brand 70 WP**Primary Reviewer:** Alison McLaughlin
For PMRA**Date:** January 22nd 2001**Secondary Reviewer(s):** Hemendra Mulye, PhD
{EPA/OECD/PMRA}**Date:** June 8, 2001**Company Code:** [For PMRA]
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EPA PC Code: 099050

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CITATION: Johnson, A.J. 1994. NI-25 Subacute Dietary Toxicity (LC50) to the Bobwhite Quail, Huntingdon Research Center Ltd. (aka. Huntingdon Life Sciences Limited), Huntingdon, Cambridgeshire, England. Report No. NPS 59/932525, Sponsor: Nippon Soda Co., Tokyo, Japan. July 21 1994. Unpublished.

Data Evaluation Report on the acute dietary toxicity of Acetamiprid insecticide to the Bobwhite Quail (*Colinus virginianus*)

PMRA Submission Number 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090
EPA MRID Number 44651860

EXECUTIVE SUMMARY:

The acute dietary toxicity of Acetamiprid (NI-25) to 10 day old Bobwhite Quail was assessed over 5 days. NI-25 was administered to two controls and two treatment groups, each with ten birds. The treatment groups received 1000 and 5000 mg a.i./kg dw (ppm) in diet respectively. Group mean food consumption was markedly depressed in the 5000 ppm treatment group; the significance of slightly decreased food consumption at the 1000 ppm level could not be ascertained due to a lack of comparative treatment levels and statistical analysis. In accordance with the US EPA Avian dietary LC₅₀ test (EPA-540/9-85-008) guideline it was not possible to verify the reported LC₅₀ > 5000 ppm because of two unexplained mortalities which occurred at the 5000 ppm treatment level (on day 2 and day 6 respectively) and the confounding problem of a lack of 95% confidence intervals. Due to a lack of comparative statistical data, the proposed 5 day NOEC value of 1000 ppm for NI-25 based on mortality also could not be confirmed. The study noted that a preliminary range finding test had been performed on groups of five birds at six dose levels (10, 50, 100, 500, 1000, and 5000), but no results were provided.

Under the conditions of this study, indirect affects on birds at the 5000 ppm dose level included significantly reduced food consumption and related depression of normal bodyweight increase. Post-mortem results for one bird dosed at the 5000 ppm level indicated abnormal muscle and fat covering. No clinical signs of direct toxicity (ie. loss of balance and co-ordination) were reported for any of the test birds. There was a slight reduction of food consumption at the 1000 ppm level and a moderate depression of normal bodyweight increase, although the statistical significance of those observations was unclear because there were only two treatment levels in the definitive test.

This toxicity study is currently classified as supplementary, but may be upgraded and reclassified as acceptable pending submission of the data set for the range finding study. In order for this study to be upgraded to acceptable, the range finding study must provide supporting evidence that the LC₅₀ is greater than 5000 ppm, and that the NOEC is 1000 ppm. If the LC₅₀ value reported in this study is supported by the range finding study results, Acetamiprid (NI-25) would be classified as practically non-toxic to Bobwhite Quail.

Results Synopsis

Test Organism: Bobwhite quail (*Colinus virginianus*), 10 days old, mean weight 13 g.

LC₅₀: >5000 mg a.i./kg diet

95% C.I.: not reported

NOEC: 1000 mg a.i./kg diet

Probit Slope: not reported

Endpoint(s) Effected: mortality

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The method followed was that given in the US EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation, Wildlife and Aquatic Organisms, Series 71-1 Avian dietary LC₅₀ test, dated Oct. 1982 and draft revised guideline dated Mar 1988.

COMPLIANCE:

It was stated that this study had been conducted according to GLP Standards under OECD Principles of GLP, OECD Environment Monograph No.45, 1992 and the US EPA, FIFRA, 40 CFR Part 160, 29 November 1983/ amended 17 August 1989. It was stated that the study also complied with the GLP standards of the UK Department of Health, the EC Council Directive and the Japan Ministry of Agriculture. Signed and dated GLP, Quality Assurance, and Signature Page were provided. There was also a signed and dated Statement of No Data Confidentiality Claim.

A. MATERIALS:

1. Test Material

NI-25

Description: Pale yellow powder

Lot No./Batch No. : NNI-03

Purity: >99 %

Stability of Compound Under Test Conditions:

Results of the analytical chemistry report (Appendix 1) indicate that NI-25 was stable at nominal concentrations of 1000 ppm and 5000 ppm in the avian diet formulation assessed over a period of 6 days during storage under animal room conditions.

Storage Conditions of Test Chemicals:

Prior to testing, NI-25 was stored at 4°C in the dark;

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the test substance analysis certificate reported that NI-25 is stable for 1 yr in the dark at 50°C, and stable for 4 yrs in the dark at -20°C.

Physicochemical properties of [test material].

| Parameter | Values | Comments |
|--------------------------|--------------|--|
| Water solubility at 20°C | not reported | * reported elsewhere as 0.4% at 25°C |
| Vapour pressure | not reported | * reported elsewhere as 1.0×10^{-6} Pa at 25°C |
| UV absorption | not reported | |
| pKa | not reported | |
| Kow | not reported | |

* These results come from the Salinity Challenge Study in this same data submission

2. Test organism:

Species: Bobwhite quail (*Colinus virginianus*)

Age at study initiation: 10 days old at the introduction of the test diet

Weight at study initiation: (mean and range): mean 13 g, range was not reported

Source: Monkfield, Bourne, Cambridgeshire, England

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A preliminary range finding test had been performed on groups of five birds at six dose levels (10, 50, 100, 500, 1000, and 5000), but no results were provided. There was no indication of how the results from the preliminary study were used to determine the conditions for the definitive study.

b) Definitive Study

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Table 1: Experimental Parameters

| Parameter | Details | Remarks |
|--|--|--|
| | | Criteria |
| <u>Acclimation</u> Period: Conditions (same as test or not): Feeding: Health (any mortality observed): | 3 days acclimation yes standard chick diet <i>ad libitum</i> none reported | acceptable <i>OECD requires at least 7 days of acclimation</i> |
| Pen size and construction materials | wooden boxes 80 x 50 x 60 cm, with approved source wood shavings as bedding, low level feeder and drinking fount | acceptable <i>EPA requires: about 35 x 100 x 24 cm; OECD requires: 300 cm² for bobwhite and 600 cm² for mallard</i> |
| Test duration | 3 days pre-treatment, 5 days treatment, 3 days post-treatment | acceptable <i>EPA/OECD requires: 5 days with treated feed and at least 3 days observation with "clean" feed.</i> |
| <u>Test concentrations</u> Nominal: Measured: | 1000 mg ai/kg diet, 5000 mg ai/kg diet 990 mg ai/kg diet, 5010 mg ai/kg diet | <i>Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC₅₀ > 5000 mg ai/kg diet. Measured conc. should be 80% of the nominal</i> |
| <u>Solvent/vehicle, if used</u> Type: Amount: | The test substance, NI-25, was added as the solid test material. | The EPA guidelines are not clear on policy for the addition of dry powder to a test diet. <i>EPA requires: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. Solvent not more than 2%.</i> |
| Diet preparation and feeding | Treated diets were prepared by mixing the test substance with the untreated basal diet. Diets were prepared one day prior to use and during the treatment period, all diets were stored in labeled polythene bags at room temperature. | <i>EPA requires: Control group tested with diet containing the maximum amount of vehicle used in treated diets</i> |
| Was detailed description and nutrient analysis of the basal diet provided | Yes. The composition of the diet was described. | acceptable |

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| Parameter (Yes/No) | Details | Remarks |
|--|---|---|
| | | Criteria |
| Indicate whether stability and homogeneity of test material in diet determined (Yes/No) | Yes. Homogeneity and stability were acceptable. | acceptable |
| Feed withholding period | none | |
| <u>Number of birds per replicate/groups</u> For negative control: For vehicle control: For treated: | 10 for each of two replicates NA 10 for each treatment level | EPA requires: 10 birds each (strongly recommended) |
| <u>Number of replicates/group (if used)</u> For negative control: For vehicle control: For treated: | 2 NA 1 at each treatment level | |
| <u>Test conditions</u> Temperature: Relative humidity (%): Photoperiod: | 25-28 °C in the animal room An infra-red heat lamp was suspended over each box to provide additional heat 29 % 12 hour | <u>Brooder temperature:</u> EPA: about 35 °C (95 °F) <u>Room temperature:</u> EPA: 22-27 °C (71-81 °F); OECD: range of 22-38 °C based on bird age and species (see OECD 205) <u>Relative humidity:</u> EPA: 30-80% OECD: 50-85% based on bird species (see OECD 205) <u>Photoperiod:</u> EPA: Minimum of 14 h of light OECD: 12-16 h of light |

b) Analytical Chemistry Report analysis for the Measured Dose Concentrations:

Table 2. Calculation of the Measured Dose

| Nominal conc. (mg ai/kg dw) (ppm): | Mean analyzed conc. (mg ai/kg dw) (ppm): | Relative mean error as measured concentration deviation from nominal | Variation of NI-25 in diet formulation samples |
|---------------------------------------|---|--|---|
| | | | |

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| Nominal conc. (mg ai/kg dw) (ppm): | Mean analyzed conc. (mg ai/kg dw) (ppm): | Relative mean error as measured concentration deviation from nominal | Variation of NI-25 in diet formulation samples |
|------------------------------------|--|--|--|
| 1000 | 990 | -1.0 % | 7.31% |
| 5000 | 5010 | +0.2 % | 1.67% |

Results of the Analytical chemistry report confirmed that the nominal concentrations of the dietary formulation were very accurate. The report confirmed that the dietary formulations were stable over a period of six days. The report also showed that the dietary formulations were homogeneous at the time of preparation, however, there were no results for homogeneity at study completion.

2. Observations:

Table 3: Observations

| Parameters | Details | Remarks |
|--|---|--|
| | | Criteria |
| Parameters measured (mortality/body weight/ mean feed consumption/ others) | mortality body weight feed consumption | <i>OECD : the mortality in the controls should not be exceed 10% at the end of the test.</i> |
| Indicate the stability and homogeneity of test chemical in the diet | Stable over 6 days Homogeneous at study initiation | |
| Indicate if the test material was regurgitated | No regurgitation was reported | |
| Treatments on which necropsies were performed | Ten control birds and ten birds from the highest treatment level were subject to macroscopic post mortem examination. | |
| Observation intervals (days) | Bodyweight: -3, 0, 5, 8 Food consumption: -3 to -1 pre-test, daily during testing (1,2,3, 4, 5), and 6 to 8 post-testing | |
| Were raw data included? | Raw analytical data, but not raw data for bodyweight, food consumption and necropsy. | |

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II. RESULTS AND DISCUSSION:

A. MORTALITY: Two birds were found dead on Days 2 and 6 respectively at the 5000 ppm treatment level. It was reported that the mortalities are not considered to be treatment related, however, no explanation was provided. It may be speculated that the mortality at day 6 was related to starvation, however, this is unlikely to be the cause of the death observed on day 2.

Table 4: Effect of NI-25 on mortality of Bobwhite quail (*Colinus virginianus*).

| Treatment (mg a.i. kg diet) | No. of birds per treatment | Mortality (Days of Study) | | | | | | | |
|--------------------------------|----------------------------------|---------------------------|---|---|---|---|---|--------|-------|
| | | -3 to -1 | 1 | 2 | 3 | 4 | 5 | 6 to 8 | Total |
| Negative control | 10 | | | | | | | | |
| Negative control | 10 | | | | | | | | |
| Test concentration 1000 | 10 | | | | | | | | |
| Test concentration 5000 | 10 | | | 1 | | | | 1 | 2 |
| LC ₅₀ | >5000 ppm | | | | | | | | |
| NOEC | 1000 ppm | | | | | | | | |

B. SUB-LETHAL TOXICITY ENDPOINTS:

There were no clinical signs of toxicity reported for any of the birds in this study. Examination of the food consumption data would, however, suggest a sub-lethal toxic effect. Food avoidance and related bodyweight may have been a factor at 1000 ppm; it appears to have been significant at the 5000 ppm level although this was not demonstrated statistically. Note that the reported values were all rounded off to the nearest gram.

Table 5: Group mean bodyweights and bodyweight changes (g)

| Treatment (mg a.i. kg diet) | No. of birds per treatment | Days of Study | | | | | | |
|--------------------------------|----------------------------------|---------------|----|----|----|---------------------|--------|--------|
| | | Bodyweight | | | | Bodyweight increase | | |
| | | -3 | 0 | 5 | 8 | -3 to 0 | 0 to 5 | 5 to 8 |
| Negative control | 10 | 9 | 13 | 19 | 25 | 4 | 6 | 6 |

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| | | | | | | | | |
|-------------------------|----|----|----|----|----|---|---|---|
| Negative control | 10 | 10 | 13 | 19 | 24 | 3 | 6 | 5 |
| Test concentration 1000 | 10 | 10 | 12 | 16 | 22 | 2 | 4 | 6 |
| Test concentration 5000 | 10 | 9 | 13 | 14 | 20 | 4 | 1 | 6 |

Table 6: Group mean food consumption (g/bird/day)

| Treatment (mg a.i. kg diet) | No. of birds per treatment | Days of Study | | | | | | | |
|--------------------------------|----------------------------------|---------------|---|---|---|---|---|--------|--------|
| | | -3 to -1 | 1 | 2 | 3 | 4 | 5 | 1 to 5 | 6 to 8 |
| Negative control | 10 | 5 | 8 | 4 | 3 | 3 | 4 | 4 | 7 |
| Negative control | 10 | 5 | 7 | 5 | 3 | 3 | 3 | 4 | 7 |
| Test concentration 1000 | 10 | 4 | 8 | 3 | 3 | 3 | 3 | 4 | 7 |
| Test concentration 5000 | 10 | 4 | 7 | 2 | 1 | 0 | 1 | 2 | 7 |

C. REPORTED STATISTICS: No statistical analysis was performed.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER: NA

E. STUDY DEFICIENCIES:

1) The US EPA protocol for the Avian Dietary LC50 Test (EPA-540/9-85-008) states that studies should be designed to establish an actual LC50 and 95% C.I.. In lieu of this, a study may demonstrate that the LC50 is greater than 5000ppm. In this case the study should show, on the basis of ten or more birds per dose, that less than one-half of the birds died at 5000 ppm. When any mortality is observed at 5000 ppm, sequentially lower doses must be tested (with ten birds per level) in order to get a dose-response series which includes at least one "no-effect" (no mortality) level.

There were two mortalities (days 2 and 6) in the 5000 ppm test group as well as another bird with abnormal fat and muscle cover identified at test termination (day 8). The proponent commented that the mortalities are not considered treatment-related but did not provide supporting evidence. While the mortality on day 6 may have been an indirect effect of starvation, the cause of the mortality on day 2 may have been directly treatment related.

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The US EPA protocol for the Avian Dietary LC50 Test (EPA-540/9-85-008) states that at a minimum, an avian dietary toxicity test will provide an LC50 with 95% confidence intervals; 95% confidence intervals were not identified in this study.

The US EPA protocol also specifies that the results from “range finding” [sic] studies may not be used to calculate the LC50, however, submission of the range finding test results might be considered helpful in interpreting the results of the definitive study.

2) The proponent should be asked to clarify what bird health and clinical signs were recorded at each observation.

F. REVIEWER'S COMMENTS: None.

G. CONCLUSIONS: This toxicity study is currently classified as supplementary, but may be upgraded and reclassified as acceptable pending submission of the data set for the range finding study which provided the basis for dose selection in the definitive study. In order for this study to be upgraded to acceptable, the range finding study must provide supporting evidence that the LC50 is greater than 5000 ppm, and that the NOEC is 1000 ppm. If the LC50 value reported in this study (> 5000 ppm) is supported by the range finding study results, Acetamiprid (NI-25) would be classified as practically non-toxic to Bobwhite Quail

III. REFERENCES:

Anderson, A., Dawe, I.S., and L. Martin. Analytical Chemistry Report. NI-25: The Analysis in Avian Diet Formulations. NPS 59/932525.

Approved 04/01/01 C.K.

Data Evaluation Report on the acute dietary toxicity of Acetamiprid insecticide to the Mallard Duck (*Anas platyrhynchos*)

PMRA Submission Number 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090

EPA MRID Number 44651861

Data Requirement: : PMRA DATA CODE: 9.6.2.5-1
EPA DP Barcode:
OECD Data Point: IIA 8.1.2
EPA Guideline: US EPA Subdivision E Guideline 71-2

Test material: NI-25 **Purity (%):** 99.57%
Common name: Acetamiprid
Chemical name: *N*¹-[(6-chloro-3-pyridyl)methyl]-*N*²-cyano-*N*¹-methylacetamidine
IUPAC: (*E*)-*N*¹-[(6-chloro-3-pyridyl)methyl]-*N*²-cyano-*N*¹-methylacetamidine
CAS name: (*E*)-*N*-[(6-chloro-3-pyridinyl)methyl]-*N*¹-cyano-*N*-methylethanimidamide
CAS No.: 160430-64-8
Synonyms: Pristine Brand RTU, Chipco Brand Tristar 70 WSP,
Adjust Brand 70 WP and Assail Brand 70 WP

Primary Reviewer: Alison McLaughlin **Date:** January 25nd 2001
For PMRA

Secondary Reviewer(s): Hemendra Mulye, PhD **Date:** June 5, 2001
{EPA/OECD/PMRA}

Company Code: [For PMRA]
Active Code: [For PMRA]
Use Site Category:[For PMRA]
EPA PC Code:

CITATION: Johnson, A.J. 1994. NI-25 Subacute Dietary Toxicity (LC50) to the Mallard Duck, Huntingdon Research Center Ltd. (aka. Huntingdon Life Sciences Limited), Huntingdon, Cambridgeshire, England. Report No. NPS 60/942075, Sponsor: Nippon Soda Co., Tokyo, Japan. July 21 1994. Unpublished.

Data Evaluation Report on the acute dietary toxicity of Acetamiprid insecticide to the Mallard Duck (*Anas platyrhynchos*)

PMRA Submission Number 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090
EPA MRID Number 44651861

EXECUTIVE SUMMARY:

The acute dietary toxicity of Acetamiprid (NI-25) to 8 day old Mallard Duck (*Anas platyrhynchos*) was assessed over 5 days in accordance with the US EPA Avian dietary LC₅₀ test (EPA-540/9-85-008). NI-25 was administered to four controls and three treatment groups, each with ten birds. The treatment groups received 200, 1000 and 5000 mg a.i./kg dw (ppm) in diet respectively. The 5 day acute dietary LC₅₀ was 5000 mg a.i./kg dw of diet (95% C.I.: 3700 - 6300 mg a.i./kg bw). The 5 day NOEC of NI-25 based on mortality was 200 mg a.i./kg dw of diet (95% C.I.: 0 - 1500 mg a.i./kg bw). According to the US EPA classification, NI-25 would be classified as slightly toxic to Mallard Duck (*Anas platyrhynchos*) on an acute dietary basis.

Under the conditions of this study, clinical signs of toxicity were noted in all birds treated at the 1000 and 5000 ppm dose levels. The birds became unsteady and imbalanced. Sublethal effects at these dose levels included significantly reduced food consumption and related depression of normal bodyweight increase. Post-mortem results for four birds dosed at the 5000 ppm level and one bird dosed at the 1000 ppm level indicated reduced muscle and subcutaneous fat.

This toxicity study is classified as supplemental and repeated because only three test concentrations were used and mortality (4 out of 10 birds) occurred at the highest test concentration (5000 ppm). The study was conducted during two different time periods. One of the test concentrations was evaluated two months after the other two test concentrations.

Results Synopsis

Test Organism: Mallard Duck (*Anas platyrhynchos*), 8 days old, mean weight 100 g.

| | |
|---|-------------------------------------|
| LC ₅₀ : 5000 mg a.i./kg diet | 95% C.I.: 3700 - 6300 mg a.i./kg bw |
| NOEC: 200 mg a.i./kg diet | 95% C.I.: 0 - 1500 mg a.i./kg bw |
| Endpoint(s) Effected: mortality | |

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The method followed was that given in the US EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation, Wildlife and Aquatic Organisms, Series 71-1 Avian dietary LC₅₀ test, dated Oct. 1982 and draft revised guideline dated Mar 1988.

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COMPLIANCE:

It was stated that this study had been conducted according to GLP Standards under OECD Principles of GLP, OECD Environment Monograph No.45, 1992 and the US EPA, FIFRA, 40 CFR Part 160, 29 November 1983/amended 17 August 1989. It was stated that the study also complied with the GLP standards of the UK Department of Health, the EC Council Directive and the Japan Ministry of Agriculture. Signed and dated GLP, Quality Assurance, and Signature Page were provided. There was also a signed and dated Statement of No Data Confidentiality Claim.

A. MATERIALS:

1. Test Material

NI-25

Description: Pale yellow powder

Lot No./Batch No. : NNI-03

Purity: >99 %

Stability of Compound Under Test Conditions:

Results of the analytical chemistry report (Appendix 1) indicate that NI-25 was stable at nominal concentrations of 200 ppm, 1000 ppm and 5000 ppm in the avian diet formulation assessed over a period of 6 days during storage under animal room conditions.

Storage Conditions of Test Chemicals:

Prior to testing, NI-25 was stored at 4°C in the dark; the test substance analysis certificate reported that NI-25 is stable for 1 yr in the dark at 50°C, and stable for 4 yrs in the dark at -20°C.

Physicochemical properties of [test material].

| Parameter | Values | Comments |
|--------------------------|--------------|--------------------------------------|
| Water solubility at 20°C | not reported | * reported elsewhere as 0.4% at 25°C |

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| Parameter | Values | Comments |
|-----------------|--------------|--|
| Vapour pressure | not reported | * reported elsewhere as $<1.0 \times 10^{-6}$ Pa at 25°C |
| UV absorption | not reported | |
| pKa | not reported | |
| Kow | not reported | |

* These results come from the Salinity Challenge Study in this same data submission

2. Test organism:

Species: Mallard Duck (*Anas platyrhynchos*)

Age at study initiation: 8 days old at the introduction of the test diet

Weight at study initiation: (mean and range): mean 100 g, range was not reported

Source: Country Game Farms, Ashford, Kent, England

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A preliminary range finding test had been performed on birds at 0, 1000 and 5000 ppm dose levels, but no results were provided. There was no indication of how the results from the preliminary study were used to determine the conditions for the definitive study.

b) Definitive Study: The definitive study was conducted in two parts. The first part included a test of two negative controls and nominal doses of 1000 and 5000 ppm. Two months later, a second portion of the study was conducted which included two controls and one nominal dose of 200 ppm. The nominal dose of 200 ppm was prepared with a methanol vehicle, however, we have no indication if two accompanying controls were prepared with methanol or if they were also "negative" untreated controls. Results for both parts of the study were combined.

Table 1: Experimental Parameters

| Parameter | Details | Remarks |
|-----------|---------|---------|
| | | |

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| Parameter | Details | Remarks |
|---|--|--|
| | | Criteria |
| Indicate whether stability and homogeneity of test material in diet determined (Yes/No) | Yes. Homogeneity and stability were acceptable. | acceptable |
| Feed withholding period | none | |
| <u>Number of birds per replicate/groups</u> | | |
| For negative control: For vehicle control: For treated: | 10 for each of four replicates NA 10 for each treatment level | EPA requires: 10 birds each (strongly recommended) |
| <u>Number of replicates/group (if used)</u> | | |
| For negative control: For vehicle control: For treated: | 4 NA 1 at each treatment level | |
| <u>Test conditions</u> | | |
| Temperature: | 21-24 °C in the animal room An infra-red heat lamp was suspended over each box to provide additional heat | <u>Brooder temperature:</u> EPA: about 35 °C (95 °F) <u>Room temperature:</u> EPA: 22-27 °C (71-81 °F); OECD: range of 22-38 °C based on bird age and species (see OECD 205) |
| Relative humidity (%): | 44 % | <u>Relative humidity:</u> EPA: 30-80% OECD: 50-85% based on bird species (see OECD 205) |
| Photoperiod: | 14 hour | <u>Photoperiod:</u> EPA: Minimum of 14 h of light OECD: 12-16 h of light |

b) Analytical Chemistry Report analysis for the Measured Dose Concentrations:

Table 2. Calculation of the Measured Dose

| Nominal conc. (mg ai/kg dw) (ppm): | Mean analyzed conc. (mg ai/kg dw) (ppm): | Relative mean error as measured concentration deviation from nominal | Variation of NI-25 in diet formulation samples |
|---------------------------------------|---|--|---|
| 200 | 192 | -4.0% | 2.32% |

Data Evaluation Report on the acute dietary toxicity of Acetamiprid insecticide to the Mallard Duck (*Anas platyrhynchos*)

**PMRA Submission Number 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090
EPA MRID Number 44651861**

| Nominal conc. (mg ai/kg dw) (ppm): | Mean analyzed conc. (mg ai/kg dw) (ppm): | Relative mean error as measured concentration deviation from nominal | Variation of NI-25 in diet formulation samples |
|---------------------------------------|---|--|---|
| 1000 | 990 | +2.0 % | 7.31% |
| 5000 | 5010 | +4.2 % | 1.67% |

Results of the Analytical chemistry report confirmed that the nominal concentrations of the dietary formulation were very accurate. The report confirmed that the dietary formulations were stable over a period of six days. The report also showed that the dietary formulations were homogeneous at the time of preparation, however, there were no results for homogeneity at study completion.

2. Observations:

Table 3: Observations

| Parameters | Details | Remarks |
|---|--|--|
| | | Criteria |
| Parameters measured (mortality/body weight/ mean feed consumption/ others) | mortality body weight feed consumption | <i>OECD : the mortality in the controls should not be exceed 10% at the end of the test.</i> |
| Indicate the stability and homogeneity of test chemical in the diet | Stable over 6 days Homogeneous at study initiation | |
| Indicate if the test material was regurgitated | No regurgitation was reported | |
| Treatments on which necropsies were performed | Ten control birds and ten birds from the highest treatment level, as well as ten birds from the lowest treatment level were subject to macroscopic post mortem examination. | |
| Observation intervals (days) | Bodyweight: -4, 0, 5, 8 Food consumption: -4 to -1 pre- test, daily during testing (1,2,3, 4, 5), and 6 to 8 post-testing | |
| Were raw data included? | Raw analytical data, but not | |

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| | | |
|--|---|--|
| | raw data for bodyweight, food consumption and necropsy. | |
|--|---|--|

II. RESULTS AND DISCUSSION:

A. MORTALITY: Two birds at the 5000 ppm treatment level were found trembling and unable to stand early in the test on Day 3; both of these birds were sacrificed on humane grounds. These birds will be considered treatment related mortalities since their described condition suggests that recovery would have been highly unlikely, not to mention consideration of the increased susceptibility to predation of any bird in this condition. Two other mortalities were noted in this group the following day. One mortality was noted at the 1000 ppm treatment level.

Table 4: Effect of NI-25 on mortality of Mallard Duck (*Anas platyrhynchos*).

| Treatment (mg a.i. kg diet) | No. of birds per treatment | Mortality (Days of Study) | | | | | | | Total |
|--------------------------------|----------------------------------|---------------------------|---|---|---|---|---|--------|-------|
| | | -3 to -1 | 1 | 2 | 3 | 4 | 5 | 6 to 8 | |
| control (for 1000, 5000 ppm) | 10 | | | | | | | | |
| control (for 1000, 5000 ppm) | 10 | | | | | | | | |
| control (for 200 ppm) | 10 | | | | | | | | |
| control (for 200 ppm) | 10 | | | | | | | | |
| Test concentration 200 | 10 | | | | | | | | 0 |
| Test concentration 1000 | 10 | | | | 1 | | | | 1 |
| Test concentration 5000 | 10 | | | | 2 | 2 | | | 4 |
| LC ₅₀ | estimated at 5000 ppm | | | | | | | | |
| NOEC | 200 ppm | | | | | | | | |

B. SUB-LETHAL TOXICITY ENDPOINTS:

There were clinical signs of toxicity such as loss of balance was reported in all birds treated at the 1000 and 5000 ppm levels. Examination of the food consumption data would suggest a sub-lethal toxic effect. Food avoidance and related bodyweight appear to have been a significant factor at the 1000 ppm and 5000 ppm levels although this was not demonstrated statistically. Note that the reported values were all rounded off to the nearest gram.

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Table 5: Group mean bodyweights and bodyweight changes (g)

| Treatment (mg a.i. kg diet) | No. of birds per treatment | Days of Study | | | | | | |
|--------------------------------|----------------------------------|---------------|-----|-----|-----|---------------------|--------|--------|
| | | Bodyweight | | | | Bodyweight increase | | |
| | | -4 | 0 | 5 | 8 | -3 to 0 | 0 to 5 | 5 to 8 |
| control (for 1000, 5000 ppm) | 10 | 51 | 101 | 218 | 311 | 50 | 117 | 93 |
| control (for 1000, 5000 ppm) | 10 | 51 | 99 | 215 | 309 | 48 | 116 | 94 |
| control (for 200 ppm) | 10 | 51 | 104 | 213 | 286 | 53 | 109 | 73 |
| control (for 200 ppm) | 10 | 51 | 97 | 203 | 278 | 46 | 106 | 75 |
| Test concentration 200 | 10 | 51 | 102 | 201 | 291 | 51 | 99 | 90 |
| Test concentration 1000 | 10 | 51 | 98 | 138 | 228 | 47 | 40 | 90 |
| Test concentration 5000 | 10 | 51 | 101 | 90 | 174 | 50 | -11 | 84 |

Table 6: Group mean food consumption (g/bird/day)

| Treatment (mg a.i. kg diet) | No. of birds per treatment | Days of Study | | | | | | | |
|--------------------------------|----------------------------------|---------------|----|----|----|----|----|--------|--------|
| | | -4 to -1 | 1 | 2 | 3 | 4 | 5 | 1 to 5 | 6 to 8 |
| control (for 1000, 5000 ppm) | 10 | 24 | 36 | 40 | 47 | 53 | 55 | 46 | 67 |
| control (for 1000, 5000 ppm) | 10 | 24 | 36 | 43 | 48 | 52 | 58 | 47 | 74 |
| control (for 200 ppm) | 10 | 26 | 39 | 35 | 48 | 59 | 60 | 48 | 62 |
| control (for 200 ppm) | 10 | 23 | 34 | 40 | 49 | 55 | 56 | 47 | 52 |
| Test concentration 200 | 10 | 30 | 39 | 45 | 46 | 54 | 59 | 48 | 60 |
| Test concentration 1000 | 10 | 23 | 18 | 28 | 31 | 35 | 37 | 29 | 62 |
| Test concentration 5000 | 10 | 24 | 10 | 14 | 17 | 19 | 21 | 15 | 57 |

C. REPORTED STATISTICS: No statistical analysis was performed. The proponent stated that it was not possible to calculate an LC₅₀ value with the data available, but that the value must

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be in excess of 5000 ppm.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER: There was no evidence that the mortalities and severe debilitation of birds at the 5000 ppm level was not treatment related. Conversely, the clinical signs of toxicity in birds treated at 1000 and 5000 ppm would suggest that the mortalities were treatment related. Statistical analysis of the limited data set for mortality was performed (Appendix I). Using trend analysis, the LC₅₀ value based on nominal dose concentrations was found to be 6176 mg ai/kg bw (C.I. 4800 - 7500 mg/kg), but this statistic was derived from only three treatment levels, including one treatment level that had been “retrofitted” into the study. Based on direct observation of the results, the actual LC₅₀ value might well have been as low as 5000 mg ai/kg bw (C.I. 3700 - 6300 mg/kg). In view of the poor data sample submitted, the more conservative LC₅₀ value of 5000 mg ai/kg bw (ppm) was selected. The proposed NOEC of 200 mg ai/kg bw (ppm) was accepted.

E. STUDY DEFICIENCIES:

1) The US EPA protocol for the Avian Dietary LC₅₀ Test (EPA-540/9-85-008) states that studies should be designed to establish an actual LC₅₀ and 95% C.I.. In lieu of this, a study may demonstrate that the LC₅₀ is greater than 5000 ppm. When any mortality is observed at 5000 ppm, however, sequentially lower doses must be tested (with ten birds per level) in order to get a dose-response series which includes at least one “no-effect” (no mortality) level.

This study clearly used an inadequate number of treatment levels which confounded calculation of a statistically sound dose-response curve. The study had no range finding test and was poorly designed. Consequently, the data includes one treatment level (200 ppm) that was “retrofitted” subsequent to test completion of the other two treatment levels. The proponent stated that the data was insufficient for the calculation of an LC₅₀ value. While it is true that the data set is not ideal, an LC₅₀ value and 95% confidence intervals could be estimated, and was estimated for the purpose of this review. A conservative estimate was made in view of the limited data set.

2) The proponent should be asked to clarify what type of controls (negative or vehicle) were used for the 200 ppm test treatment level.

F. REVIEWER'S COMMENTS:

This toxicity study is classified as supplemental and repeated because only three test concentrations were used and mortality (4 out of 10 birds) occurred at the highest test concentration (5000 ppm). The study was conducted during two different time periods. One of the test concentrations was evaluated two months after the other two test concentrations. Also,

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methanol was used as a carrier, which is generally not accepted by EPA.

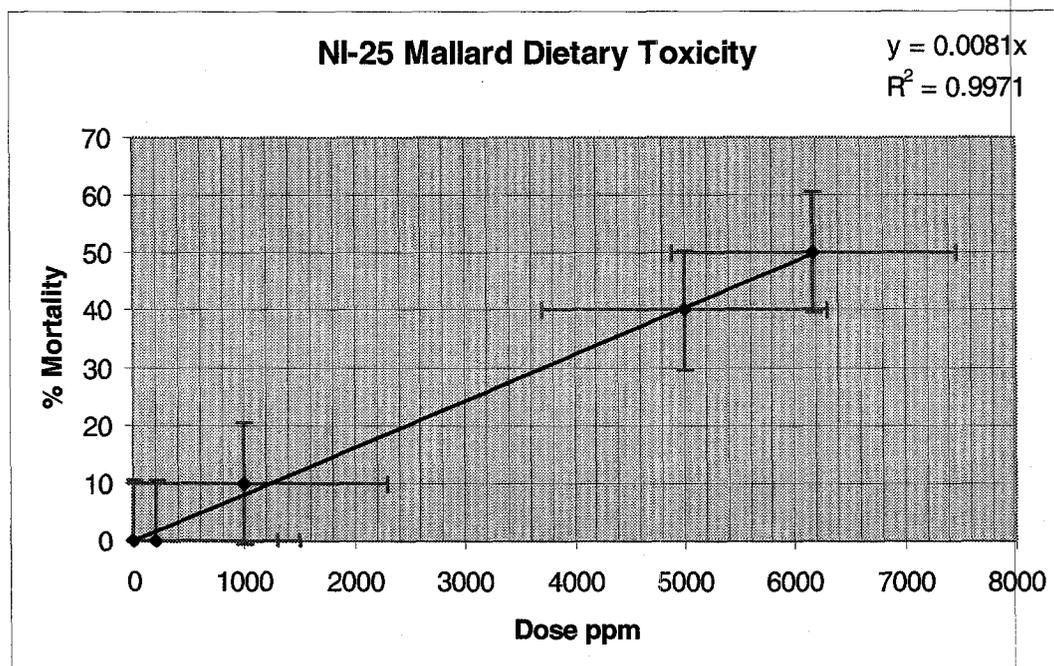
G. CONCLUSIONS: This toxicity study is classified as supplemental. The LC₅₀ value was found to be 5000 mg ai/kg bw (C.I. 3700 - 6300 mg a.i./kg bw). The NOEC value was 200 mg ai/kg bw (C.I.: 0 - 1500 mg a.i./kg bw). On the basis of these results, Acetamiprid (NI-25) would be classified as slightly toxic to Mallard Duck. There were sub-lethal clinical signs of toxicity, such as loss of balance, reported in all birds treated at the 1000 and 5000 ppm levels.

III. REFERENCES:

Anderson, A., Dawe, I.S., and L. Martin. Analytical Chemistry Report. NI-25: The Analysis in Avian Diet Formulations. NPS 59/932525.

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Appendix I:

Limited statistics were performed on the available data. There were only three treatment levels and a control level from which to extrapolate.

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NOEC and LC₅₀ values were extrapolated through a trend line. A conservative estimate of the LC₅₀ was made in view of the limited data set. Using Excel software. Standard error values were calculated and are shown in red. The equation for the slope of the line appears next to the title.

Accordingly

LC₅₀: 5000 mg a.i./kg bw 95% C.I.: 3700 to 6300 mg a.i./kg bw
NOEC: 200 mg a.i./kg 95% C.I.: 0 to 1500 mg a.i./kg bw

Approved 04/01/01 C.K.